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Morphological characterization and diversity of endomycorrhizae in the rhizosphere of Carob tree (*Ceratonia siliqua*) in Morocco

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ABSTRACT

The diversity of mycorrhizal fungi in the rhizosphere of the carob tree (*Ceratonia siliqua*) was studied in five regions of Morocco: Taroudant, Khénifra, Afourar, Nador and Ksiba. Microscopic examination of the carob tree soil, collected from the studied areas, revealed the presence of 31 AM fungal species belonging to six genera *Glomus* (16 species), *Acaulospora* (7 species), *Scutellospora* (4 species), *Gigaspora* (2 species), *Entrophospora* and *Pacispora* (one species each). The genus *Glomus* is the most abundant with a distribution percentage of 93%. Species richness varies between 13 and 18 species. All the encountered species were morphologically characterized basing on: shape, color, average size of the spore and its wall, spore surface and size of the hyphae. The diversity of AM fungi in different ecological studied zones varies from one site to another, it is higher in Taroudant site, the Shannon-Wiener index H ' is in the order of 2.78 and the index of Margalef IM 3.6. Furthermore, the similarity index of Jaccard shows a strong similarity between the Taroudant site and that of Afourar.

Keywords: *Ceratonia siliqua*, endomycorrhizae, Carob tree, Morocco, Fungi.

1. INTRODUCTION

Biodiversity refers, according to Ramade (1993), to the number of living species that inhabit a particular place (in the ecosystemic sense, etc). It includes all the life forms, ecosystems and ecological processes; it recognizes the hierarchy at the genetic level, taxon and ecosystems (Boudouresque, 2014). Microbial diversity, on the other hand, includes the diversity of bacteria, protozoa, fungi, and unicellular algae. It is the most extraordinary life reservoir in the biosphere (Nirmalnath, 2010). Diversity is composed of two elements, wealth and regularity, so that the greatest diversity occurs in communities with the presence of many different species (wealth) with equal abundance relativity (regularity) (Huston, 1994). In Morocco, biodiversity is one of the richest in the world because of the geographical position of Morocco between Europe and Africa, also the great spatial variability of climatic and geological conditions increases this wealth, heterogeneous and complex of ecosystems and habitats (Chillasse *et al*., 2001), ranging from high mountains covered with forests and snow to the desert confines apparently almost azoic, passing through the vast alluvial plains, rivers, lakes and marine waters.

The Moroccan fauna structure shows a predominance of terrestrial phanerogams with about 4,500 species. Fungi and lichens are also well represented respectively by 820 and 700

species reported (Ait Aguil, 2005). Multicellular algae, including nearly 700 species, with 489 macro algae and nearly 200 species belonging to phytoplankton (Dahssi *et al*., 2004). However, erosion and desertification phenomena cause annually a loss of about 31,000 hectares of land. To counter these problems, considerable efforts are devoted to reforestation (10 to 15 000 ha / year), by the use of indigenous and endemic plant species that can improve the success rate of reforestation operations (Konate, 2007). The carob tree (*Ceratonia siliqua*) is one of these species, it is largely distributed in Morocco as spontaneous or planted stands (Emberger, 1938; Ouchkif, 1988). The carob tree is an agro-forestry-pastoral species with enormous socio-economic and ecological interests (Batlle and Tous, 1997; Gharnit *et al*., 2001). It has also the ability to establish a symbiotic association with AM fungi (El Asri *et al*., 2014), association that allows a better plant nutrition (mainly phosphorus) especially in arid and semi-arid environments, improve the aggregation and stability of soil (Rillig and Mummey, 2006) and protect plants against pathogens (Newsham *et al*., 1995). Mycorhization of *Ceratonia siliqua* seedlings is therefore an interesting path to explore for the restoration of the sclerophyllous forest which the original environment presents difficult conditions for the relocation of plant cover (Ammari *et al*., 2006). For the valuation of forest species of agronomic interest, it is necessary to give special attention to the endomycorhizal fungi diversity (AMF) at the root zone of the carob tree growing in different regions of Morocco. In this sense, the present work is a continuation of the work of El Asri *et al*. (2014) on *Ceratonia siliqua* endomycorrhizae. Indeed, few exhaustive studies have been conducted in Morocco on the diversity of AMF associated with carob tree.

2. MATERIALS AND METHODS

2.1 Site selection

To cover the main populations of the carob tree in Morocco, surveys were conducted in five regions (Taroudant, Khenifra Afourar, Nador and Ksiba) from east to south-west of Morocco. The selected sites cover the main structural formations of the Middle Atlas, East and West Rif and the Anti-Atlas. In each region, five sites were selected for soil sampling in the rhizosphere of the carob tree. Oriental and occidental Rif and the Anti-Atlas. In each region, five sites were selected for soil sampling in the rhizosphere of the carob tree. Soil samples were taken randomly from five trees per site (2 kg of ground / tree) at a depth of 0-20 cm.

2.2 Spores extraction

Spores were extracted following the wet sieving method described by Gerdemann and Nicolson (1963). In a 1 L beaker, 100 g of each composite soil sample is submerged in 0.5 L of tap water and stirred for 1 minute with a spatula. After 10 to 30 seconds of settling, the supernatant was passed through four sieve superimposed with decreasing mesh (500, 200, 80 and 50 microns). This operation is repeated twice. The content retained by the sieve of 200, 80 and 50 microns is divided into two tubes and centrifuged for 4 min at 9000 rev / min. The supernatant is discarded and a viscosity gradient is created by adding 20 ml of sucrose solution of 40% to each centrifuge tube (Walker *et al*., 1982). The mixture was rapidly stirred and the tube is returned again in the centrifuge for 1 min at 9000 rev / min. Unlike the first centrifugation step, the supernatant is poured into the sieve with a mesh of 50 microns, the resulting substrate was rinsed with distilled water to remove the sucrose, then disinfected with an antibiotic solution (streptomycin). The spores are then recovered with a little distilled water in a flask.

2.3 Diversity Analysis

Spores identification:

Conventional techniques for the identification of AM fungi are based on the morphology of the spores. This approach is long and difficult to control the characteristics of spores, especially the wall, structure and morphology are the most important criteria for identification of AM fungus (Morton and Bentivenga, 1994).

Species richness (S):
The specific richness S is represented by the total or average number of species per unit area. This index can be used to analyze the taxonomic structure of the settlement, it also helps to distinguish spatial variations: rich areas and poor areas.
S = number of species in the study area

Margalef index:
The Margalef index or index of biodiversity Margalef is a measure used to estimate the biodiversity of a community based on the digital distribution of individuals of different species depending on the individual's number in the sample (Margalef, 1958).
I_M = (S-1) / Ln N
Where:
S = number of species present
N = the total number of individuals found (belonging to all species).
Ln = neperian logarithm of a number.

Shannon-Wiener Index:
The Shannon index is used to express the diversity taking account the number of species and abundance of individuals within each species. Thus, a community dominated by one species will have a lower coefficient than a community in which all species are co-dominant. The value of the index ranges from 0 (one species, or a species that largely dominates all others) to Ln S (when all species have the same abundance) (Shannon and Weaver, 1949).

$$H' = -\sum_{i=1}^{s}\left[\left(\frac{ni}{N}\right)\ln\left(\frac{ni}{N}\right)\right]$$

Where:
S = species total number;
ni = number of species individuals in the sample;
N = total number of individuals of all species in the sample.

Jaccard similarity index:
The Jaccard similarity index measures the difference in diversity between two sites, it is 1 in case of complete similarity (that is if the two sites have the same species) and 0 if the sites are dissimilar (the case where both sites have no species in common) (Jaccard, 1912).

$$Cj = j / (a + b - j)$$

j = the number of species found in both sites
a = the number of species in the site A
b = the number of species in the site B

3. RESULTS

The AM fungi isolated from the rhizosphere of the carob tree (*Ceratonia siliqua*) developing in different ecological zones (Afourar, Ksiba Khénifra, Taroudant, Nador) were identified to species level based on the spores' morphological characteristics (Table 1). The specific richness of the studied sites is given in Table 2. This wealth varies from one site to another, Taroudant and Nador recorded the highest number of species, respectively 18 and 17 species, Ksiba, 15 species. The lowest number was recorded at the Afourar and Khénifra areas, with 13 species.

The set of species found in different sites belong to six genera: *Glomus* (16 species), *Acaulospora* (7 species) *Scutellospora* (4 species), *Gigaspora* (2 species), *Entrophospora* and *Pacispora* (one species each). The abundance of these fungal species is given in Table 3. In the Afourar region, *Glomus versiforme* and *Acaulospora Gedanensis* are the most abundant with respectively 13 and 12 spores / 100g of soil, in the area of Ksiba *Scutellospora nigra* (14 spores / 100 g of soil) is more abundant, followed by *Glomus clarum* and *Glomus etunicatum* (12 spores / 100g soil). In twenty-five sampling points *Glomus etunicatum* is distributed in fifteen sampling point with a percentage of 60%, followed by *Scutellospora nigra* in ten sampling points (40%). *Glomus clarum*, *Glomus aggregatum* and *Acaulospora Gedanensis* are recorded in nine points (36%).

Furthermore, *Acaulospora* sp.2, *Acaulospora* sp.1, *Acaulospora* sp.3, *Acaulospora* sp.4, *Acaulospora denticulate*, *Glomus chimonobambusae*, *Glomus* sp.3, *Glomus* sp.4, *Glomus minutum* and *Scutellospora castanea* are found only in one sampling point, with a percentage of 4%. Furthermore, *Acaulospora* sp.1, *Acaulospora* sp.2, *Acaulospora* sp.3, *Acaulospora* sp.4, *Acaulospora denticulate*, *Glomus chimonobambusae*, *Glomus* sp.1,

Table 1: Identification of mycorrhizal fungi isolated from the rhizosphere of *Ceratonia siliqua* in the different study sites.

S.N.	Form	Color	Average size (µm)	Wall size (µm)	Spore surface	Size of hyphae	Name
1	globular	dark yellow	130	10.4	smooth	-	*G. macrocarpum*
2	globular	clear yellow	65	5.2	smooth	41.6	*A. gedanensis*
3	Oval	Clear yellow	64	5	smooth	-	*A. denticulata*
4	globular	yellow	58	3.75	smooth	134.5	*A. laevis*
5	globular	clear yellow	67	5.2	granular	52	*G. aggregatum*
6	Oval	Brown	59.8	2.6	granular	-	*E. infrequens*
7	Oval	yellow	75.4	5.2	smooth	-	*Gi. decipiens*
8	globular	yellow	44.2	2.6	smooth	36.4	*A. gedanensis*
9	Ellipsoid	yellow	72.8	2.6	smooth	36.4	*A. laevis*
10	Globular	dark yellow	106.6	2.6	smooth	-	*G. margarita*
11	Globular	yellow	59.8	2.6	smooth	31	*Gi. decipiens*
12	Ellipsoid	dark brown	104	7.8	smooth	-	*G. macrocarpum*
13	Globular	yellow	44.2	2.6	smooth	36.4	*A. gedanensis*
14	Globular	yellow	72.8	5.2	smooth	59.8	*A. laevis*
15	Globular	dark brown	78	10.4	smooth	10.4	*G. aggregatum*
16	Globular	brown	130	10.4	smooth	-	*G. deserticola*
17	Globular	yellow	65	7.8	smooth	-	*G. aggregatum*
18	Globular	yellow	60	2.6	smooth	40	*G. aureum*
19	Oval	clear brown	77	7	smooth	-	*G. macrocarpum*
20	Ellipsoid	yellow	41.6	2.6	smooth	13	*A. gedanensis*
21	Ellipsoid	dark yellow	54.6	5.2	smooth	-	*G. aggregatum*
22	Oval	yellow	53	4	smooth	19	*G. aureum*
23	Oval	dark yellow	48	4	granular	13	*G. clarum*
24	Subglobular	clear yellow	49.4	6.25	granular	-	*G. chimonobambusae*
25	Globular	yellow	109.6	2.6	granular	-	*G. clarum*
26	Globular	brown	48	2.6	smooth	-	*G. deserticola*
27	Oval	dark yellow	52	5.2	smooth	44.2	*G. aggregatum*
28	Oval	clear brown	67	5	smooth	-	*G. aggregatum*
29	Oval	yellow	53	4	smooth	-	*G. aureum*
30	Globular	clear yellow	88	5.2	granular	28	*G. clarum*
31	Globular	brown	48	2.6	smooth	-	*G. deserticola*
32	Globular	Yellow	54	2.6	smooth	-	*G. etunicatum*
33	Ellipsoid	dark yellow	49	2.6	granular	26	*G. etunicatum*
34	Oval	dark yellow	90	5.2	granular	-	*G. aggregatum*
35	Globular	Clear yellow	83	8	smooth	-	*G. macrocarpum*

….Table 1: Identification of mycorrhizal fungi isolated from the rhizosphere of *Ceratonia siliqua* in the different study sites.

S.N.	Form	Color	Average size (µm)	Wall size (µm)	Spore surface	Size of hyphae	Name
36	Oval	dark yellow	91	7.8	smooth	-	*G. aggregatum*
37	Oval	dark yellow	39	2.6	granular	46.8	*G. aggregatum*
38	Oval	dark yellow	40	2.66	granular	80	*G. clarum*
39	Globular	clear yellow	51	3	smooth	-	*G. aureum*
40	Oval	dark yellow	61	8	irregular	32	*G. macrocarpum*
41	Globular	dark yellow	50	5.6	smooth	-	*G. etunicatum*
42	Globular	clear yellow	122	8	granular	-	*G. fasciculatum*
43	Ellipsoid	dark yellow	48	5.3	granular	40	*G. geosporum*
44	Ellipsoid	clear yellow	46	2,6	smooth	-	*G. etunicatum*
45	Globular	clear yellow	91	10	granular	48	*G. macrocarpum*
46	Oval	dark yellow	80	5.2	granular	160	*G. clarum*
47	Globular	yellow	56	2.6	granular	120	*G. clarum*
48	Ellipsoid	clear yellow	59	6	smooth	-	*G. macrocarpum*
49	Ellipsoid	clear yellow	85	3	granular	50	*G. intraradices*
50	Globular	clear brown	43	2.6	granular	-	*G. geosporum*
51	Globular	clear yellow	45	5	smooth	-	*G.etunicatum*
52	Subglobular	clear yellow	111	10	smooth	-	*G. etunicatum*
53	Ellipsoid	clear yellow	63	3	granular	95	*G. intraradices*
54	Oval	yellow	106	2.6	granular	-	*G. clarum*
55	Oval	clear yellow	59	10	smooth	21	*G. macrocarpum*
56	Oval	clear brown	82	2.6	granular	85	*G. geosporum*
57	Ellipsoid	clear yellow	80	3	granular	-	*G. intraradices*
58	Subglobular	clear yellow	58	4	smooth	40	*G. etunicatum*
59	Globular	clear yellow	61	2.6	irregular	-	*G. intraradices*
60	Globular	clear yellow	61	2.6	irregular	-	*G. intraradices*
61	oval	clear yellow	77	8	granular	160	*G. monosporum*
62	Globular	clear yellow	40	2.6	smooth	44	*G. mosseae*
63	Globular	clear yellow	82	2.6	granular	76	*G. claroideum*

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S.N.	Form	Color	Average size (µm)	Wall size (µm)	Spore surface	Size of hyphae	Name
64	Globular	clear yellow	133	8	granular	-	*G. versiforme*
65	Globular	clear yellow	48	5	granular	40	*G. macrocarpum*
66	Ellipsoid	dark yellow	50	5.3	granular	45	*G. geosporum*
67	Subglobular	yellow	93	5	granular	-	*G. etunicatum*
68	Oval	dark yellow	45	5	smooth	13	*G. macrocarpum*
69	Globular	clear yellow	42	4	smooth	50	*G. geosporum*
70	Oval	yellow	34	2.6	granular	160	*G. monosporum*
71	Globular	yellow	77	5	granular	160	*G. mosseae*
72	Globular	yellow	80	5	smooth	-	*G. claroideum*
73	Oval	clear yellow	70	5	granular	-	*G. versiforme*
74	Globular	yellow	85	10	smooth	-	*P. robiginia*
75	Oval	dark yellow	61	2.6	irregular	-	*S. castanea*
76	Globular	yellow	74	5.3	granular	-	*S. fulgida*
77	Globular	yellow	72	4	granular	-	*G. versiforme*
78	Globular	clear yellow	53	5	smooth	45	*G. mosseae*
79	Globular	yellow	64	4	smooth	-	*P. robiginia*
80	Globular	yellow	40	2.6	smooth	16	*S. heterogamma*
81	Globular	noire	122	2.6	smooth	122	*S. nigra*
82	Globular	yellow	45	5	smooth	-	*G. macrocarpum*
83	Oval	yellow	88	5	smooth	-	*G. macrocarpum*
84	Globular	clear yellow	40	4	smooth	24	*G. etunicatum*
85	Globular	clear yellow	53	5	granular	18	*G. macrocarpum*
86	Globular	clear yellow	101	10	smooth	80	*G. etunicatum*
87	Globular	yellow	106	2.6	granular	40	*G. clarum*
88	Oval	yellow	45	5	smooth	32	*G. etunicatum*
89	Globular	clear brown	48	2.6	irregular	-	*S. castanea*
90	Globular	yellow	114	5.3	smooth	-	*G. macrocarpum*
91	Globular	clear Yellow	59	2.6	smooth	37	*G. aureum*
92	Globular	clear Yellow	48	4	smooth	18	*S. heterogamma*
93	Globular	clear brown	48	5	granular	-	*G. macrocarpum*
94	Globular	clear Yellow	93	8	irregular	-	*G. intraradices*
95	Globular	brown	101	8	smooth	-	*G. etunicatum*

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S.N.	Form	Color	Average size (µm)	Wall size (µm)	Spore surface	Size of hyphae	Name
96	Oval	clear brown	59	2.6	irregular	-	*S. castanea*
97	Globular	clear yellow	80	5.3	smooth	106	*G. geosporum*
98	Oval	clear yellow	40	5	smooth	30	*G. etunicatum*
99	Oval	clear brown	51	2.6	irregular	-	*S. castanea*
100	Oval	yellow	82	2.6	granular	-	*G. intraradices*
101	Oval	clear yellow	88	3	smooth	-	*Acaulospora sp1*
102	Oval	yellow	91	2.6	granular	-	*G. geosporum*
103	Globular	yellow	43	2.6	smooth	53	*G. etunicatum*
104	Oval	yellow	43	2.6	smooth	48	*G. geosporum*
105	Globular	yellow	50	5	smooth	18	*S. heterogamma*
106	Globular	dark brown	37	2.6	smooth	42	*S. nigra*
107	Globular	hyaline	146	5	smooth	-	*G. minutum*
108	Globular	hyaline	70	3	smooth	13	*G. minutum*
109	Oval	clear yellow	40	4	smooth	-	*G. etunicatum*
110	Globular	dark yellow	111	5	granular	-	*G*. versiforme
111	Oval	dark yellow	61	2.6	smooth	53	*G. monosporum*
112	Oval	clear Yellow	90	5	smooth	-	*G .intraradices*
113	Globular	dark brown	77	5	granular	-	*G. macrocarpum*
114	Globular	clear brown	90	8	smooth	-	*G. macrocarpum*
115	Globular	clear Yellow	156	13	granular	-	*G. versiforme*
116	Globular	clear Yellow	93	3	granular	-	*Acaulospora* sp.2

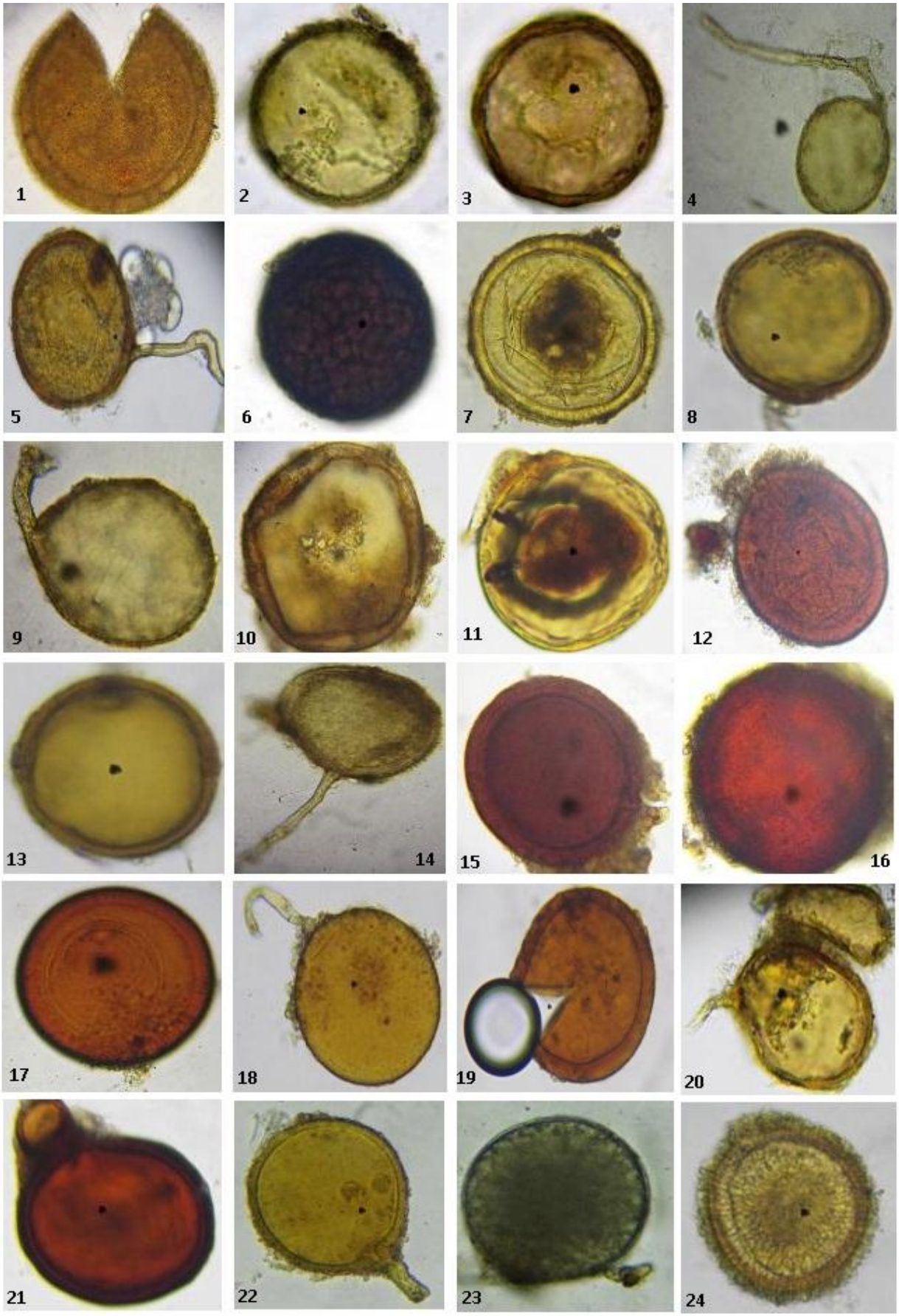
Note :
A – Acaulospora ; *E – Entrophospora ;* Gi – *Gigaspora* ; G – *Glomus ; P - Pacispora ; S – Scutellospora*

Glomus sp.2, *Glomus minutum* and *Scutellospora castanea* are found only in one sampling point, with a percentage of 4%. The distribution of different types of AM fungi in different ecological zones studied is presented in Table 5. The genus *Glomus* is the most frequent, it is present in 23 points, and the distribution percentage is 92%, followed by *Scutellospora* (15 points) and *Acaulospora* (14 points), each with a distribution percentage of 60 and 56%.

Gigaspora genus and *Entrophospora pacispora* are less frequent with a low distribution percentage, 4, 3 and 2% respectively.

The AM fungi diversity in different ecological zones studied varies from one site to another (Table 6). The greatest diversity was found in the region of Taroudant, the number of AMF species is 18. The Shannon diversity index is higher in Taroudant area (H '= 2.78), followed

Figure 1: Mycorrhizal fungi species isolated from the rhizosphere of *Ceratonia siliqua*.



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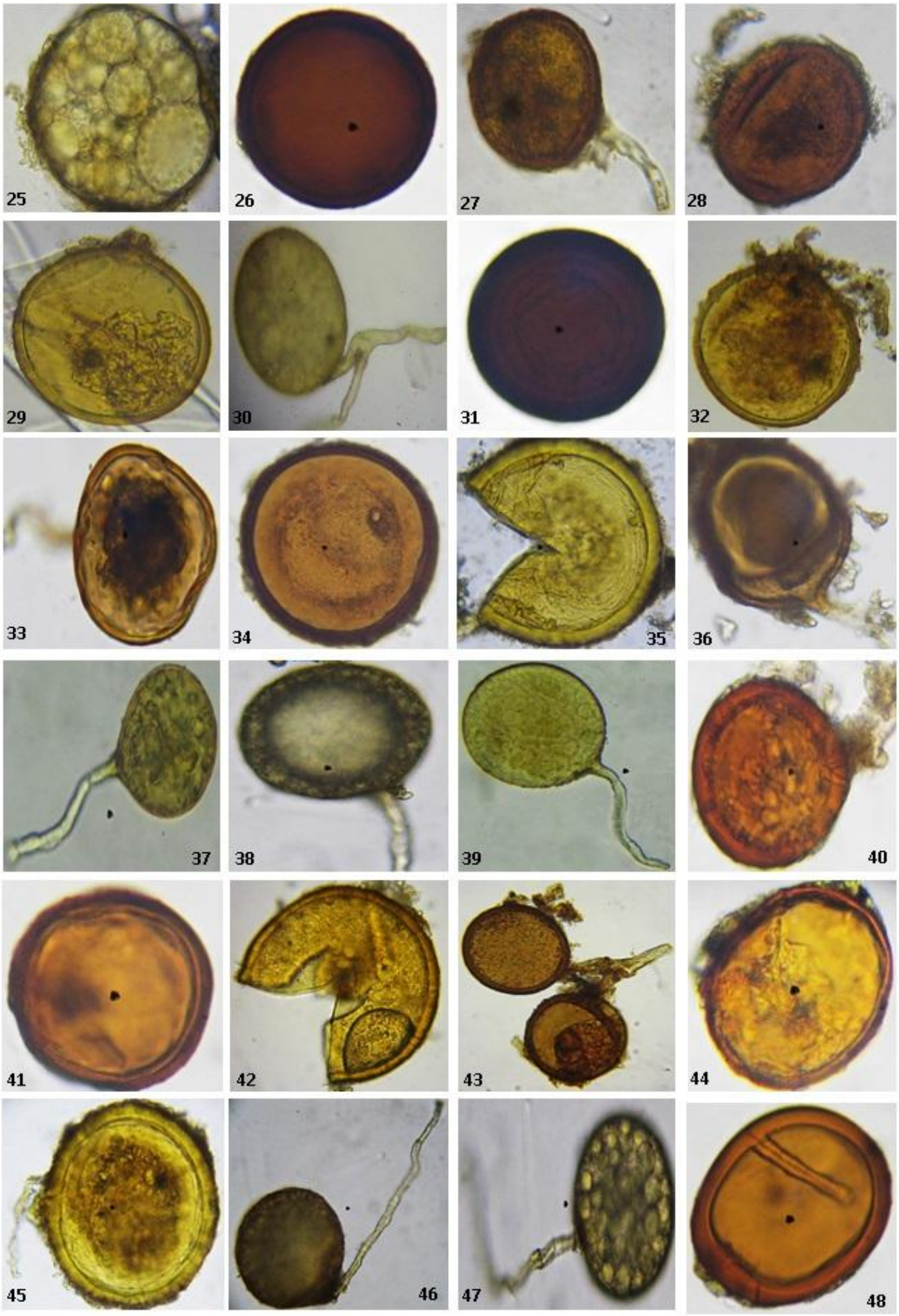

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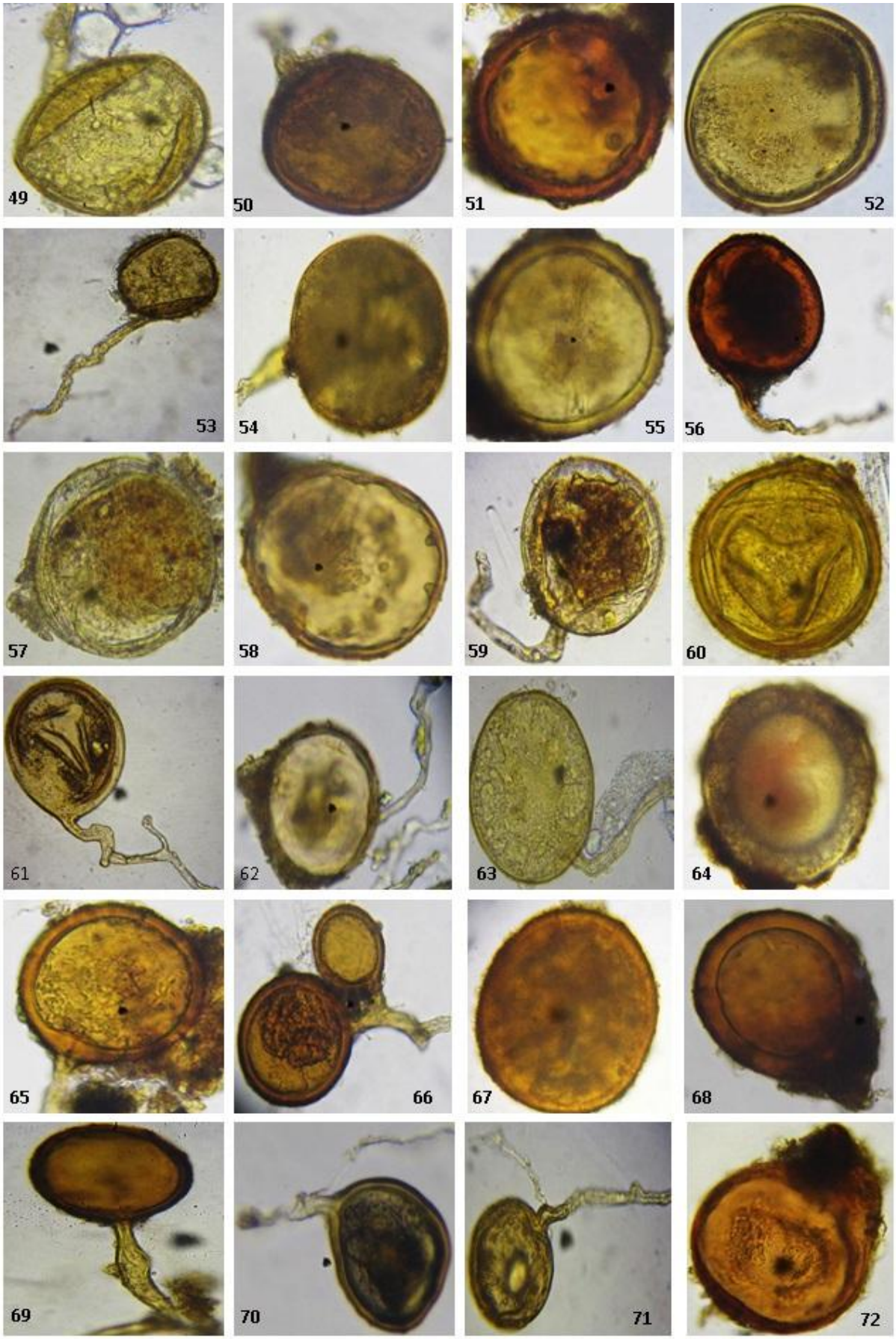

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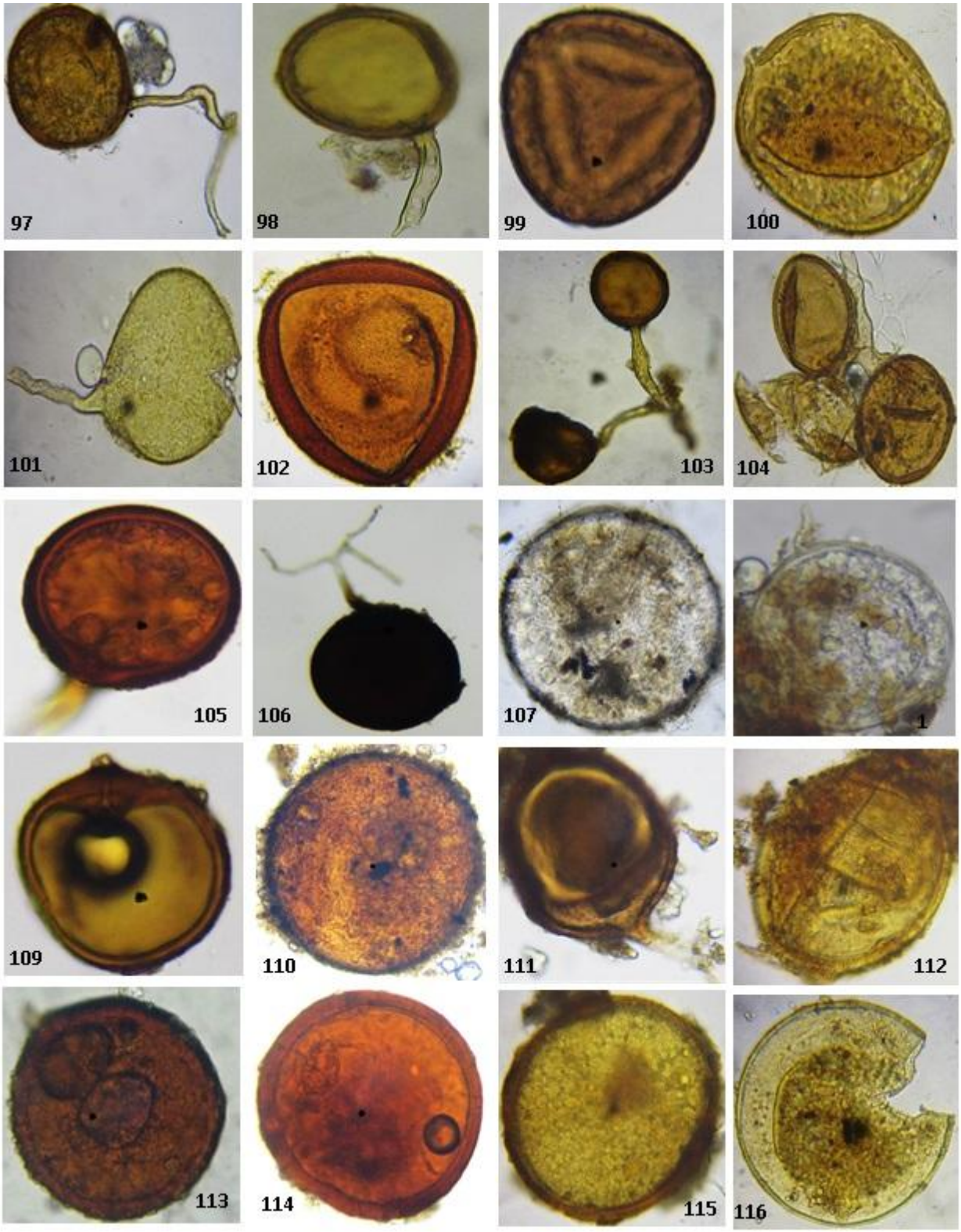

Table 2: AM fungi species present in the different study areas.

Mycorrhizal species	Number of spores per 100g of soil				
	Afourar	Ksiba	Khénifra	Taroudant	Nador
A. laevis	4	-	-	9	9
A. gedanensis	12	9	12	-	19
Acaulospora sp.1	-	-	3	-	-
Acaulospora sp.2	-	-	-	-	2
Acaulospora sp.3	-	-	-	2	-
Acaulospora sp.4	-	-	-	3	-
A. denticulate	-	-	5	-	-
E. infrequens	4	4	-	3	-
G. decipiens	3	4	-	-	-
G. margarita	-	-	-	-	6
G. aggregatum	5	8	12	6	13
G. aureum	6	10	-	-	-
G. chimonobambusae	-	-	3	-	-
G. clarum	-	12	5	5	23
G. deserticola	-	4	-	8	8
G. etunicatum	11	12	20	11	13
G. fasciculatum	4	-	-	6	-
G. geosporum	5	8	5	8	-
G. intraradices	5	-	5	4	15
Glomus sp. 1	-	-	-	-	2
Glomus sp. 2	-	-	-	3	-
G. macrocarpum	-	11	7	12	7
G. minutum	-	3	-	-	-
G. monosporum	-	8	-	-	10
G. mosseae	-	-	-	10	6
G. versiforme	13	-	7	8	9
P. robiginia	-	-	-	3	4
S. castanea	-	4	-	-	-
S. fulgida	-	-	-	4	5
S. heterogamma	4	9	4	-	-
S. nigra	8	14	8	6	9
TOTAL	**84**	**120**	**96**	**111**	**160**

by that of Nador (H' = 2.67). Khénifra zone has recorded the lowest Shannon Index (2.39) compared to other sites. The Margalef index is also higher in the Taroudant region (3.60), followed by the one calculated in the Nador area (3.15). The lowest species richness was recorded at the Khénifra zone (2.4).

The Jaccard similarity index of AM fungi in all study sites are presented in Table 7. A strong similarity was found between the two sites of Taroudant and Afourar (0.69), also low similarity was recorded between the two sites of Taroudant and Ksiba.

DISCUSSION

The rhizospheric soil analysis of the carob tree showed the existence of a diverse and very extensive community of mycorrhizal fungi. Indeed, in this study, up to 31 arbuscular fungal species belonging to six genera were isolated and identified. The highest AM fungus richness has been recorded in the Taroudant region (18), followed by Nador (17), and the lowest number of species was recorded in Afourar areas and Khénifra (13). Ouahmane *et al*. (2012) isolated from the rhizosphere of the carob tree, at Ourika Valley, seven AM fungus species, belonging to the genera *Glomus* and *Gigaspora*. Tchabi *et al*. (2008) found that species richness of AMF in

natural forests is higher than in agricultural fields. Undisturbed forest land (Leal *et al.*, 2009; Tchabi *et al.*, 2008 and Shi *et al.*, 2007), grass lands (Oehl *et al.*, 2003) and desert plants (Stutz, 2003) are rich in AM fungi species than agricultural land (Oehl *et al*., 2003).

Table-4. Frequence and distribution Percentage of AM fungi in all the sites studied (25 sampling points).

Mycorrhizal species	Distribution Frequency	Distribution percentage (%)
Acaulospora laevis	5	20
Acaulospora gedanensis	9	36
Acaulospora sp.1	1	4
Acaulospora sp.2	1	4
Acaulospora sp.3	1	4
Acaulospora sp.4	1	4
Acaulospora denticulate	1	4
Entrophospora infrequens	3	12
Gigaspora decipiens	2	8
Gigaspora margarita	2	8
Glomus aggregatum	9	36
Glomus aureum	4	16
Glomus chimonobambusae	1	4
Glomus clarum	9	36
Glomus deserticola	5	20
Glomus etunicatum	15	60
Glomus fasciculatum	2	8
Glomus geosporum	7	28
Glomus intraradices	6	24
Glomus sp.1	1	4
Glomus sp.2	1	4
Glomus macrocarpum	8	32
Glomus minutum	1	4
Glomus monosporum	3	12
Glomus mosseae	3	12
Glomus versiforme	6	24
Pacispora robiginia	2	8
Scutellospora castanea	1	4
Scutellospora fulgida	2	8
Scutellospora heterogamma	4	16
Scutellospora nigra	10	40

Glomus is the most common genera, it is present in 15 points from 25 sampling points, with a high distribution percentage (92%). This dominance was reported in several studies in Latin America (Lopes *et al*., 1983; Cruz, 1989) southwest of Ethiopia (Muleta *et al*., 2008; Jefwa *et al*., 2009) , dry African mountain forests of Ethiopia (Tesfaye *et al*., 2004), rainforest Xishuangbanna in China (Zhao *et al*., 2001), tropical rainforest in Mexico (Guadarrama and Alvarez-Sanchez, 1999) arid and semi-arid areas of northern Jordan (Mohammad *et al*., 2003), coastal dunes (Nicolson *et al*., 1979 Giovannetti *et al*., 1983;

Table 5: Frequency and distribution percentage of AM fungi types in all study sites (25 sampling points).

Genres	Distribution frequency	Distribution (%)
Acaulospora	14	56
Entrophospora	3	12
Gigaspora	4	16
Glomus	23	92
Pacispora	2	8
Scutellospora	15	60

Bergen *et al*., 1984; Schenck *et al*., 1980; Ragupathy, 1998; Hatimi and Tahrouch 2007) and Moroccan Tetraclinis (Abbas *et al*., 2006). Different authors have associated the dominance of *Glomus* with its ability to produce more spores in a shorter time than other genres such as *Gigaspora* and *Scutellospora* (Bever *et al*., 1996) and also to its adaptability to drought and soil salinity (Haas and Menge, 1990; Blaszkowski *et al*., 2002).

Glomus etunicatum is the most dominant species, it is present in 15 stations from 25 sample, with a high distribution percentage (60%). Lakshmipathy *et al*. (2004) reported the dominance of *Glomus etunicatum* in the rhizosphere of Cashew. However, Lakshmipathy (2005) Mohankumar and Mahadevan (1987) noted that some environmental factors such as soil pH, temperature, moisture, organic matter and the physical and chemical property of soil have an important role in the distribution of AM fungi species. The diversity of AM fungi in the

different study areas varies from one site to another, it is higher in Taroudant, where the diversity index of Margalef (3.60) and Shannon diversity index (2.78) are higher, this great diversity could be attributed to the fact that this region is characterized by a forest canopy, marked by the presence of argan tree (*Argania spinosa*) much wider and roughly dense and fruit farming dominated by olive groves and citrus mainly located along the Oued Souss in the direction of Ouled Berhil toward east (Hanane, 2010). The AMF diversity is based on the presence of a high diversity in plant species (M. N. Abubacker *et al.*, 2014 and Oehl *et al*., 2003).

Table 6: AM fungi diversity in all study sites.

Ecological zones	Species numbers (S)	Total number (N)	Margalef diversity index	Shannon diversity index
Afourar	13	84	2.7	2.44
Ksiba	15	122	2.91	2.62
Khénifra	13	96	2.4	2.39
Taroudant	18	112	3.60	2.78
Nador	17	160	3.15	2.67

On the other hand the diversity and composition of AM fungal communities vary depending on the types of habitats around the world (Opik *et al*., 2008). Helgason *et al.* (2007) found a high diversity of AM fungi in tropical forests compared to other ecosystems. Several studies have shown that the intensity of use and cropping system greatly influence the diversity and AM fungi communities (Oehl *et al*., 2003). Other studies conducted in five regions of the Swiss Alps have shown that AM fungi communities vary also with altitude (Oehl *et al*., 2011).

Table 7: Jaccard similarity index of AM fungi in all study sites.

	Afourar	Ksiba	Khnifra	Taroudant	Nador
Afourar		0.47	0.44	0.69	0.43
Ksiba			0.4	0.32	0.33
Khénifra				0.34	0.36
Taroudant					0.52
Nador					

CONCLUSION

The concept of AM fungi diversity in forest ecosystems is dependent on several factors (plant formations, soil type, the climate or microclimate factors...). However, AM fungi are specific to soils and ecosystems that react sensitively to the mode and the exploitation intensity. On the other hand, the diversity of mycorrhizal fungi naturally occurring in carob soils can be selected and used in reforestation and restoration of degraded ecosystems and even to improve the production of vigorous carob plants. Indeed, the controlled mycorrhiza is a powerful tool in organic farming practices inscribed in the sustainable land management.

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